CLAIMS

1. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing a single-stranded nucleic acid having plural partial and sequential base sequences to be detected (A-strand) and a single-stranded nucleic acid having a base sequence complementary to a base sequence of the A-strand (B-strand);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing a nucleic acid having a sequence complementary to a partial and sequential base sequence within the region between a 3'-end of the A-strand and the base sequence to be detected which is located nearest the 3'-end as a primer for elongating the B-strand;

(4) performing PCR reactions using the A-strand and B-strand as templates, and using the primers immobilized on the substrate, and the primer for elongating the B-strand;

(5) forming a hybridized product of a nucleic acid corresponding to the A-strand which has been

elongated and amplified as a result of the PCR reactions and bound to the substrate and a nucleic acid corresponding to the B-strand which has been elongated and amplified and has not bound to the substrate; and

(6) detecting the base sequence to be detected by detecting the hybridized product in the respective primer-immobilized regions in the array.

2. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing a single-stranded nucleic acid having plural partial and sequential base sequences to be detected (A-strand) and a single-stranded nucleic acid having a base sequence complementary to a base sequence of the A-strand (B-strand);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing a nucleic acid having a partial and sequential base sequence within the region between a 5'-end of the A-strand and the base sequence to be detected which is located nearest the 5'-end as a primer for elongating the A-strand and

preparing a nucleic acid having a sequence complementary to a partial and sequential base sequence within the region between a 3'-end of the A-strand and the base sequence to be detected which is located nearest the 3'-end as a primer for elongating the B-strand;

(4) performing PCR reactions using the A-strand and B-strand as templates, and using the primers immobilized on the substrate, the primer for elongating the A-strand, and the primer for elongating the B-strand;

(5) forming a hybridized product of a nucleic acid corresponding to the A-strand which has been elongated and amplified as a result of the PCR reactions and bound to the substrate and a nucleic acid corresponding to the B-strand which has been elongated and amplified and has not bound to the substrate; and

(6) detecting the base sequence to be detected by detecting the hybridized product in the respective primer-immobilized regions in the array.

3. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic acids each having a partial and sequential base sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded

nucleic acids each having a base sequence complementary to a base sequence of each strand of the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing nucleic acids each having a sequence complementary to a partial and sequential base sequence within the region between a 3'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strands (PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);

(4) performing PCR reactions using each strand of the A-strand group and each corresponding strand of B-strand group as templates, and using the primers immobilized on the substrate, and the plural primers for elongating the B-strands of the PB-strand group;

(5) forming a hybridized product of a nucleic acid corresponding to the A-strand group which has been elongated and amplified as a result of the PCR reactions and bound to the substrate and a nucleic

acid corresponding to the B-strand group which has been elongated and amplified and has not bound to the substrate; and

(6) detecting the base sequence to be detected by detecting the hybridized product in the respective primer-immobilized regions in the array.

4. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic acids each having a partial and sequential base sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded nucleic acids each having a base sequence complementary to a base sequence of each strand of the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing nucleic acids each having a partial and sequential base sequence within the region between a 5'-end of each strand of the A-strand group and the base sequence to be detected

which is located nearest the 5'-end as primers for elongating the A-strands (PA-strand group: PA1-strand to PAn-strand: $n \geq 2$) and preparing nucleic acids having a sequence complementary to a partial and sequential base sequence within the region between a 3'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strands (PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);

(4) performing PCR reactions using each strand of the A-strand group and each corresponding strand of the B-strand group as templates, and using the primers immobilized on the substrate, the primers for elongating the A-strands of the PA-strand group, and the primer for elongating the B-strand of the PB-strand group;

(5) forming a hybridized product of a nucleic acid corresponding to the A-strand group which has been elongated and amplified as a result of the PCR reactions and bound to the substrate and a nucleic acid corresponding to the B-strand group which has been elongated and amplified and has not bound to the substrate; and

(6) detecting the base sequence to be detected by detecting the hybridized product in the respective primer-immobilized regions in the array.

5. A method of detecting a nucleic acid

according to any one of claims 1 to 4, further comprising a step of washing and removing a reaction solution on the substrate after the PCR reactions.

6. A method of detecting a nucleic acid according to any one of claims 1 to 4, wherein the primer for elongating the B-strand is labeled, and the hybridized product is detected using the label.

7. A method of detecting a nucleic acid according to claim 5, wherein the label is a fluorescent dye.

8. A method of detecting a nucleic acid according to claim 7, further comprising a step of observing the fluorescent dye using a confocal fluorescent microscope for detecting the hybridized product.

9. A method of detecting a nucleic acid according to any one of claims 1 to 4, wherein the hybridized product is detected using a fluorescent dye as an intercalator or a groove binder which interacts with a double-stranded nucleic acid.

10. A method of detecting a nucleic acid according to claim 9, further comprising a step of observing the fluorescent dye using a confocal fluorescent microscope for detecting the hybridized product.

11. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing a single-stranded nucleic acid having plural partial and sequential base sequences to be detected (A-strand) and a single-stranded nucleic acid having a base sequence complementary to a base sequence of the A-strand (B-strand);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing a nucleic acid having a sequence complementary to a partial and sequential base sequence within the region between a 3'-end of the A-strand and the base sequence to be detected which is located nearest the 3'-end as a primer for elongating the B-strand;

(4) performing PCR reactions using the A-strand and the B-strand as templates, and using the primers immobilized on the substrate, and the primer for elongating the B-strand, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label

incorporated in the nucleic acid.

12. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing a single-stranded nucleic acid having plural partial and sequential base sequences to be detected (A-strand) and a single-stranded nucleic acid having a base sequence complementary to a base sequence of the A-strand (B-strand);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing a nucleic acid having a partial and sequential base sequence within the region between a 5'-end of the A-strand and the base sequence to be detected which is located nearest the 5'-end as a primer for elongating the A-strand and preparing a nucleic acid having a base sequence complementary to a partial and sequential base sequence within the region between a 3'-end of the B-strand and the base sequence to be detected which is located nearest the 3'-end as a primer for elongating the B-strand;

(4) performing PCR reactions using the A-strand

and the B-strand as templates, and using the primers immobilized on the substrate, the primer for elongating the A-strand, and the primer for elongating the B-strand, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label incorporated in the nucleic acid.

13. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic acids each having a partial and sequential base sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded nucleic acids each having a base sequence complementary to a base sequence of each strand of the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing nucleic acids each having a sequence complementary to a partial and sequential base sequence within a region between a 3'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strands (PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);

(4) performing PCR reactions using each strand of the A-strand group and each corresponding strand of the B-strand group as templates, and using the primers immobilized on the substrate and the plural primers for elongating the B-strands of the PB-stand group, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label incorporated in the nucleic acid.

14. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic acids each having a partial and sequential base sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded nucleic acids each having a base sequence complementary to a base sequence of each strand of

the A-strand group (B-strand group: B1-strand to Bn-strand: n ≥ 2);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing nucleic acids each having a partial and sequential base sequence within the region between a 5'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 5'-end as primers for elongating the A-strands (PA-strand group: PA1-strand to PAn-strand: n ≥ 2) and preparing nucleic acids each having a base sequence complementary to a partial and sequential base sequence within the region between a 3'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strand (PB-strand group: PB1-strand to PBn-strand: n ≥ 2);

(4) performing PCR reactions using each strand of the A-strand group and each corresponding strand of the B-strand group as templates, and using the primers immobilized on the substrate and respective

primers of the PA-strand group and PB-strand group, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label incorporated in the nucleic acid.

15. A method of detecting a nucleic acid according to any one of claims 11 to 14, further comprising a step of washing and removing a reaction solution on the substrate after the PCR reactions.

16. A method of quantitative determination of a nucleic acid based on signals detected according to any one of claims 1 to 4 and 11 to 14.

17. A method of detecting a nucleic acid according to any one of claims 11 to 14, wherein the label is a fluorescent dye.

18. A method of detecting a nucleic acid according to claim 17, further comprising a step of observing the fluorescent dye using a confocal fluorescent microscope for detecting the hybridized product.

19. A method of detecting a nucleic acid according to any one of claims 1 to 4 and 11 to 14, wherein at least the PCR reactions and nucleic acid detections are performed in a form in which the

primer arrays are present in the same container.

20. A method of detecting a nucleic acid according to claim 19, wherein the respective PCR reactions and nucleic acid detections are performed while observing intermittently using the same means.

21. An apparatus for detecting a nucleic acid, which enables the method of detecting a nucleic acid according to claim 19, comprising:

a PCR reaction container; and

detection means.

22. An apparatus for detecting a nucleic acid according to claim 21,

wherein said PCR container comprises a substrate having a surface with immobilized polymers, a reaction chamber and a temperature controlling unit,

wherein said substrate is transparent against wavelength used for detection

wherein said reaction chamber is facing to said surface,

wherein said temperature controlling unit is placed at a position not preventing operation of said detection means, and

wherein said detection means is placed on the side opposite to said surface in relation to said substrate.

23. A kit for detecting a nucleic acid, comprising a primer array; a PCR reaction reagent;

and a nucleic acid detecting reagent, for performing the method according to any one of claims 1 to 4 and 11 to 14.

24. A kit for detecting a nucleic acid according to claim 23, wherein the nucleic acid detecting reagent is a fluorescent dye serving as an intercalator or groove binder which acts on a double-stranded nucleic acid.